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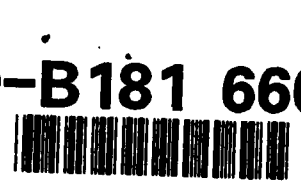

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The natural bioluminescence of the microscopic marine dinoflagellate alga, Pyrocystis lunula, is quenched measurably in the presence of toxins. This principle, trade-named Lumitox, was employed in the development of a portable, hand-held instrument trade-named TOX-BOX, in response to a solicitation by the United States Army for a rapid field toxicity test for water supplies. TOX-BOX is capable of detecting dangerous concentrations of toxins in most environmental media.

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Unchecked items are not applicable.

Arthur V. Stiffey

Principal Investigator's Signature

Arthur V. Stiffey

February 28, 1994

Date

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No graduate degrees resulted from this research.

RAPID FIELD TOXICITY TEST FOR WATER SUPPLIES

FINAL REPORT (CONTINUITY PERIOD): PROJECT TOX-BOX

February 28, 1994

INTRODUCTION

Under U.S. Department of Defense Small Business Innovative Research Topic Number A92-163, the U.S. Army Medical Research Acquisition Activity solicited for a portable, hand-held "Rapid Field Toxicity Test for Water Supplies." In response to the contractor's proposal to design such a device (Project TOX-BOX), the Army awarded a six-month contract No. DAMD 17-93-C-3093. This contract expired August 16, 1993. In addition to regular monthly reports, the contractor submitted a Final Report dated August 15, 1993. The contents of that report, which outlined the Lumitox bioluminescent principle and explained in detail all the work performed during those six months, need not be repeated here.

Because the contractor had carried design of its portable tester to a primitive piece of hardware (TOX-BOX) that worked marginally well, the Army approved a four-month extension to the original contract. The Army also had become interested in an isolation procedure (Project Lucifer) that would substitute feeze-dried luciferin and luciferase to produce light, in place of the living organism. These compounds, which are responsible for generation of light by the organism, would make TOX-BOX much more portable.

By the December 16, 1993 expiration of the extended contract period, the contractor had produced a much-improved TOX-BOX but had not yet tested it. The contractor also had developed a culture of *Pyrocystis lunula*, the test organism, but had not begun the isolation

procedure. This Final Report will cover the four-month extended contract period during which that work was accomplished.

Subsequent to the December 16, 1993 expiration of the extended contract, the ex-contractor continued work on both projects. Now there are two TOX-BOXes that are as sensitive and precise as the old laboratory apparatus, and a preliminary isolation has been effected. In view of the Army's interest, and pursuant to the ex-contractor's appreciation of the Army's support, a gratuitous report on this work is included here.

WORK DESCRIPTION

Month Seven

During Month Seven (the first month of the extended contract period), the contractor made little tangible progress. Printed circuitry, thought to be necessary for TOX-BOX sensitivity, was received, returned for corrections, and again received for installation. Malfunctions in the laboratory apparatus were corrected. A local refrigeration manufacturer began design of an environmental chamber for cultivation of the test organism. The Fifth District Federal Court chose this time to call the Principal Investigator to jury duty. Work began on a cooperative research project with Baroid Drilling Fluids, Inc. to test oil-well drilling muds (Project Mud); this was thought to be relevant to testing water supplies, particularly muddy sources.

Month Eight

Because building an incubator locally would take too long, the contractor bought a ready-made model from Fisher Scientific. It was necessary to install fluorescent tubes to satisfy the

organism's diurnal light requirement (it's a plant). This incubator turned out to be a good choice, easily maintaining the required 20 degrees C. Culture population was insufficient to begin Project Lucifer. Testing of TOX-BOX MKI #2 was a disappointment. Up to this time, reliance had been placed on photodiodes. It appears that not only are they lacking in power (TOX-BOX had gone from two to twelve for increased aspect and amplification), but also their sensitivity peak is in the red band rather than at the pale green light emitted by the excited organism. The "cleaner" printed circuit could not make up for this deficiency, and the amplification required for sensitivity resulted in a very unstable output. It was decided to return to a photomultiplier tube that is tuned to the correct wavelength; during the course of Project TOX-BOX, such a tube had become available. Project Mud continued.

Month Nine

The culture continued to propagate but still was too sparse to harvest for Project Lucifer. TOX-BOX was under reconstruction, employing a miniature photomultiplier tube, the original hand-wired circuit, and a stirrer. It was decided that TOX-BOX hand shaking did not transfer enough of the shear needed to stimulate light production. Project Mud was nearly completed.

Month Ten

The culture was ready for harvesting at the end of Month Ten. Much of the expensive lab equipment necessary for Project Lucifer had been ordered and some had been received. TOX-BOX MKI #3 was nearly complete. Project Mud was completed with excellent results. Sensitivity of the lab apparatus was tested against sodium lauryl (dodecyl) sulphate (SDS), the prescribed calibration substance for the EPA-mandated toxicity testing protocol that uses *Mysid* shrimp.

Mysid LC_{50} (half of them die) for SDS is about 10 ppm. Lumitox EC_{50} (half the light is quenched) for SDS is about 4 ppm.

Postscript

Expiration of the Army contract on December 16, 1993 did not stop Lumitox Gulf (San'Doil's environmental affiliate) from working. Testing of TOX-BOX MKI #3 was a complete success. Lumitox now has two TOX-BOXes, with built-in stirrers, that are as sensitive and precise as the lab apparatus and are no larger than a building brick. They have jacks to produce hard copy on a strip-chart recorder; stirrer timers; and nickel-cadmium batteries rechargable by 110-volt wall current, which is used in the lab. TOX-BOX production cost is estimated at $1000, the photomultiplier tube accounting for about half the expense. The PI has succeeded in isolating and freeze-drying a crude extract of luciferin. At this writing, using ammonium sulphate, he also has precipitated protein that contains luciferase, as confirmed by the Bradford technique. However, continuation of Project Lucifer depends on financial support. Some of this progress, plus Project Mud, is chronicled in Lumitox Gulf's 1994 Offshore Technology Conference paper, "Portable, Accurate Toxicity Testing," a pre-publication copy of which is attached for the Army's information.

CONCLUSIONS

During the original six-month contract period, the contractor completed design and fabricated a hand-held, portable toxicity tester (TOX-BOX MKI) that could discriminate between toxic and non-toxic substances. That version used a hand-wired circuit and two photodiodes. In an effort to improve sensitivity, the contractor built TOX-BOX MKI #2, which used a printed

circuit and up to twelve photodiodes; this version was hopelessly unstable. Sensitivity was not improved, primarily because the photodiode's sensitivity peak is in the red band, instead of the pale green light emitted by the excited organism. During a four-month extended contract period, efforts to improve this model were unsuccessful. The contractor redesigned the tester and built (but did not test) TOX-BOX MKI #3. This model uses a recently available miniature photomultiplier tube tuned to light green, and returned to the simpler hand-wired circuit. Also during this period, the contractor formulated plans and began to acquire equipment for Project Lucifer, the isolation of luciferin and luciferase: the compounds that enable the organism to produce light. The purpose of Project Lucifer is to make TOX-BOX conveniently portable.

Immediately subsequent to the contract period, the now ex-contractor proved TOX-BOX MKI #3 to be as sensitive and precise as the Lumitox lab apparatus. Estimated initial production cost is $1000 per unit; the photomultiplier tube represents about half the expense. A crude luciferin extract has been freeze-dried, and precipitation by ammonium sulphate of a crude protein containing luciferase has been confirmed by the Bradford technique. Continuation of Project Lucifer depends on further financing. Establishing yield parameters and setting up a production pilot plant would be an appropriate SBIR Phase II project. Because the isolation principle will have been demonstrated and some of the expensive equipment already has been acquired, a Phase II budget might be significantly lower now than the $750,000 originally proposed.

PERSONNEL

Arthur V. Stiffey: Vice President, Lumitox Gulf, Principal Investigator

Thomas G. Nicolaides: Electronics Designer and Technician

Robert W. Sabate': President, San'Doil Exploration L.C.; Technical Writer

Edmund L. Dewailly: President, Lumitox Gulf L.C.; Laboratory Assistant

No graduate degrees resulted from this research.

APPENDIX

ABSTRACT

Ever tightening environmental regulations, severe penalties for non-compliance, and expensive remediation costs have stimulated development of methods to detect and measure toxins. Most of these methods are bioassays that must be performed in the laboratory; none previously devised has been truly portable. The U.S. Army, through the Small Business Innovative Research program, has developed a hand-held, field deployable unit for testing toxicity of battlefield water supplies. This patented system employs the measurable quenching, in the presence of toxins, of the natural bioluminescence produced by the marine dinoflagellate alga *Pyrocystis lunula*.

The procedure's inventor used it for years to measure toxicity concentrations of chemical warfare agents - actually, their simulants, primarily in the form of pesticides and herbicides - plus assorted toxic reagents, waterbottom samples, drilling fluids, even blood. While the procedure is more precise, cheaper, and faster than most bioassays, until recently it was immobile. Now it is deployable in the field.

The laboratory apparatus has been proven to be sensitive to toxins in concentrations as low as a few parts per billion, repeatable within a variation of 10% or less, and - unlike some other bioassays - effective in turbid or colored media. The laboratory apparatus and the hand-held tester have been calibrated with the EPA protocol that uses the shrimplike *Mysidopsis bahia*. The test organism tolerates transportation well, but must be rested a few hours at the test site for regeneration of its light-producing powers. Development of a freeze-dried reagent, now under way, will make the portable tester even more convenient to use.

Toxicity now can be measured confidently in soils, water columns, discharge points, and many other media *in situ*. Most significant to the oil industry is that drilling fluids can be monitored continuously on the rig.

BACKGROUND

A quick, precise, novel bioassay that uses the

References and illustrations at end of paper

natural bioluminescence of the microscopic marine dinoflagellate *Pyrocystis lunula* was developed by a U.S. Navy scientist to detect and measure toxicity of, among other substances, chemical warfare agents. This work was conducted with CWA simulants, mostly in the form of commercial herbicides and pesticides. The inventor and associates disclosed the novel method to the scientific community in a 1985 report on the photometer used in the procedure (1) and in a 1986 paper on the detection of trichothecenes (2). The trichothecenes paper described a perfect qualitative correlation (Fig. 1) of the novel test with the Environmental Protection Agency protocol (3) that uses the tiny, shrimplike *Mysidopsis bahia* for testing drilling fluids toxicity. The novel method was announced to the petroleum industry as a development study in a 1990 Offshore Technology Conference paper (4). This work led to five Navy patents (5), to which a private environmental company has acquired exclusive marketing licenses under the government's technology transfer program.

Since OTC 1990, the novel toxicity testing method has undergone considerable development. A 1992 paper (6) reporting on a joint U.S.-U.S.S.R. study of waterbottom sediments collected from the Bering Straits and the Chukchi Sea showed perfect correlation of the novel method with a bioassay that uses the brine shrimp *Artemia salina*.

Also in 1992, a three-way cooperative research project among the Navy, its private licensee, and Louisiana State University (7) firmly established the theoretical basis of the novel method, and demonstrated its excellent correlation in the higher toxicity ranges both with the *Mysid* shrimp test and with a commercial bioassay that uses bioluminescent bacteria.

In 1993, the private licensee, under a Small Business Innovative Research contract with the U.S. Army, developed a portable, hand-held toxicity tester for use with battlefield water supplies. This unit is nearly as sensitive as its much larger laboratory counterpart. It uses the live organism, but development of a freeze-dried reagent is underway.

Most recently, a cooperative research project between the private licensee and a commercial drilling fluids company established an excellent correlation of the novel method with the *Mysid* shrimp test, using fresh drilling fluids of controlled composition. This is the first publication of that effort.

PROCEDURE

A detailed description of the novel procedure appeared in the OTC 1990 paper (4) and cannot be repeated in its entirety here. Briefly, *P. lunula* (Fig. 2) is cultured in Fernbach flasks in an environmental chamber at 20 degrees C under a 12-hour on/off light cycle. Population grows to 2000 cells / ml. Culture medium is artificial seawater plus certain vitamins, minerals, and a pH buffer. Cells in 1 ml of the culture are counted microscopically with a Sedgwick-Rafter chamber and a hand clicker. The medium is diluted appropriately to 100 cells / ml and 3 ml are placed in 20-ml clear glass scintillation vials with a Cornwall automatic syringe.

Samples usually are run in quadruplicates. The first four contain only the culture medium as a control; if a large number of samples are to be run, control replicates are interspersed. With an Eppendorf automatic pipette, the sample replicates are inoculated with 10, 25, and 50 ul sample substrates. All vials are placed in the dark incubator for four hours to allow toxins to react with the cells, and for the cells to regain their light-producing powers.

The vials then are placed in a carousel (Fig. 3). Each in turn is stirred vigorously for one minute; the shearing action stimulates the cells to make light. This work must be done in the dark. The light is converted by a photomultiplier tube into an electric current, which flows through the patented light-integrating circuit (Fig 4) and thence to a strip-chart recorder, where it is depicted as a cumulative curve (Fig. 5).

The presence of toxins in a sample quenches (inhibits) light, as compared to the control: if its curve is half as high, inhibition is 50%; a quarter as high, 75%. Each set of replicates is averaged (X) and its standard deviation (SD) computed. Relative SD (RSD) is SD / X. RSDs for control and mildly toxic samples typically run 5-10%. A strongly toxic sample RSD usually is higher, about 10-15%, because of its numerically lower base, despite lower SDs. Quality control requires average RSD (sum of all RSDs / number of replicate sets - usually four, including control) to be less than 15%; otherwise, the samples are rerun.

Sample medium pH is strongly buffered to 7.6. Because sample substrates are so small a fraction of the total sample (1/60 to 1/300), pH and salinity are not significantly affected. Therefore, either marine or freshwater samples can be run without adjustment. This also minimizes turbidity. However, turbidity does not seem to be a problem. Samples rendered opaque with amido black dye yield light values not significantly different from clear samples.

LSU STUDY

Louisiana State University (7), through their Petroleum and Environmental Engineering departments, performed the first rigorous quantitative toxicity tests on drilling fluids. LSU had collected a large number of mud samples that had been assayed with the *Mysid* shrimp test. Many were non-toxic, and none exceeded the EPA LC50 limit of 30,000 ppm. LSU tested 60 of these with the novel method, 26 of which also had been tested with bioluminescent bacteria.

Mud testing uses the suspended particulate phase (SPP, Fig. 6). LSU sample concentrations were 100% SPP plus dilutions to 10% and 1%. Response curves have a characteristic hyperbolic shape (Fig. 7) that LSU likens to enzymatic response. One of the organism's light-producing compounds is an enzyme. Adaptation of the Michaelis-Menken enzyme reaction kinetic model yields the formula:

$$Q(S) = \frac{QmaxS}{Kd + S}$$

where:

Q(S) = light quenching at S
S = substrate concentration
Qmax = maximum quenching

Kd represents the concentration of a toxic sample at which half the light is quenched, a concept similar to LC50. In practice, the inventor had observed that for most substances tested, the boundary between tolerable and dangerous toxicity was 50% quenching. The reaction curve, from which Kd is computed or measured, can be approximated from at least two points: one on the climbing limb and one on the Qmax portion of the curve. As will appear below, this is not always convenient to achieve. Although LSU's selected criterion is Kd, the private licensee finds test values in the Qmax range to be more practical predictors of toxicity as measured by other assays.

Fig. 8 shows results of the novel test on 11

of the drilling fluids. Correlation of 30 novel tests with *Mysid* shrimp test results (Fig. 9) shows good agreement with LC50s below about 350,000 ppm. Correlation with the bioluminescent bacteria assay (Fig. 10) is good below an EC50 (half light reduction) of about 200,000 ppm. In both cases, this is in the high toxicity range, which of course is of prime interest to an operator.

Poor correlation at low concentrations could have been aggravated by inter-lab variation and elapsed time from the shrimp tests (up to a year) and from the bioluminescent bacteria tests (up to six months). Samples were field muds of unknown composition. LSU contends that sample toxicity tends to increase with time, while the drilling fluids company that participated in the tests described below suggested the opposite. In either case, toxicity stability is a problem in studies of this kind. For these reasons, the private licensee decided to test fresh muds of known composition.

MUD TESTS

The private licensee equipped a mud-testing lab of its own and asked a prominent drilling fluids company to prepare some mud samples across the entire range of toxicity, and to subject them to its *Mysid* shrimp test. Six samples were forwarded, of which five were unknowns. Mud #1 was described as a generic, non-toxic mud that served as an additional control. Actually, all but Mud #2, a field mud of unknown composition, were composed of the same generic mud mixed with various additives. Lab construction delayed novel testing for a few weeks after receipt of the samples. A few interesting surprises emerged from these tests, one of which was due to deterioration of one of the samples. Repeat testing demonstrated stability of the other muds. All were tested at 10, 25, and 50 ul substrate levels, indicated in the table of results (Fig. 11) as E10, etc.

Graphic results of the novel testing method are shown on Fig. 12. Relative toxicity is quite clear, although the E10 and E25 values are not in perfect agreement. Correlation of E50 with LC50 is shown on Fig. 13. Order of toxicity is in perfect agreement except for Mud #4, "overkilled" with potassium chloride - KCl.

Mysid shrimp are very sensitive to K; the bioluminescent bacteria reportedly are not - they tend to agree with the novel method. Under the assumption that the presence of drilling mud is irrelevant, various concentrations of KCl were tested in water. *P. lunula* turns out to be completely insensitive to KCl dissolved in the artificial seawater medium, which itself contains 1.64% KCl. The KCL drilling mud test, however, had yielded significant but low toxicity (36% quenching). There could be some synergistic effect with the "non-toxic" generic mud, which alone quenched 15% of the light. KCl is not very toxic. It has been used by people as a NaCl substitute, as an electrolyte replenisher, and in larger doses as a purgative. However, in order to compete with the *Mysid* shrimp test, the private licensee is developing a K-concentration test by color-comparator chart.

Mud #3 originally tested as very mild. Designed as a lubricant and for stuck drill pipe, it contains a vegetable-base ester that is very toxic. Unlike the traditional lubricant it replaces, diesel oil (whose toxicity is stable), the ester is designed to be very unstable. The original sample had grown a gel, which when stained and examined microscopically could be seen to contain several varieties of bacteria. The ester had been biodegraded in a matter of weeks by oleophyllic organisms. A freshly mixed sample generated the most toxic results of the lot.

SDS TEST

EPA requires periodic testing of *Mysid* shrimp to demonstrate stable toxicity tolerance. The prescribed test substance is sodium lauryl (dodecyl) sulphate (SDS). Informal reports by labs to the private licensee of ultrapure SDS LC50s ranged from 6 to 12 ppm.

Fig. 14 shows SDS EC50 of *P. lumula* to be 3.7 ug / ml, or 3.7 ppm wt / vol, about three times as sensitive as the *Mysid* shrimp.

PORTABLE TESTER

In 1993, the private licensee was awarded a Small Business Innovative Research contract by the U.S. Army to design a hand-held, portable unit to test toxicity of battlefield water supplies. Although design details cannot be disclosed at this time, the Army project resulted in such a device (photo, Fig. 15). Dimensions are 19 x 11 x 6 cm topped by a 7-cm sample chamber (a much smaller version now is under construction). Output is digital LED. Sensitivity testing was conducted with 3 ml of culture media at different population levels: 100, 50, and 25 cells / ml. Because light production is proportionate to cell count, this approach mimics light quenching by toxins. Results from quintuplicate testing are shown on Fig. 16.

Again, although SD at low-light levels is smaller than at high levels, the smaller base value yields a portable-unit RSD a little higher than equivalent highly toxic values do on the lab equipment. Otherwise, precision and sensitivity appear comparable.

TOXICITY CRITERIA

At this point, the private licensee feels that it has developed accurate criteria for at least rangefinder assessment of drilling fluids toxicity. Bearing in mind research experience and variation potential on the order of 10%, light-quenching percentages at the E50 level correspond to the EPA *Mysid* shrimp protocol as follows:

Q%	Toxicity	Indication
15	detectable	non-dangerous
50	marginal	potentially dangerous, monitor carefully
60	toxic	dangerous, approaching EPA limit
70	very toxic	dangerous, exceeds EPA limit

DRY REAGENT

The portable unit uses the living organism. *P. lumula* tolerates transportation well at moderate temperatures, but because its light-producing powers are sapped both by light and by motion, it must be rested in the dark for a few hours after arrival at the sampling station.

Prior to his retirement from the Navy and his affiliation with the private licensee, the inventor had proposed a project to develop a freeze-dried reagent in order to enhance portability. Under a negotiated Navy contract, the private licensee now is conducting that research.

OTHER APPLICATIONS

The private licensee currently is negotiating contracts to test such diverse substances as anti-cancer drugs, all of which are highly toxic, and oil-well produced waters, most of which are not. The novel method also is useful for testing point-source discharges, hazardous waste leacheates, agricultural chemical contamination, wetlands pollution, subsurface toxic plumes, and marine anti-fouling paints. It offers potential of replacing animals for

testing cosmetics, drugs, and other substances designed for human or animal consumption.

CONCLUSION

A novel toxicity testing procedure that uses the natural bioluminescence of the microscopic marine dinoflagellate, *Pyrocystis lunula*, is faster, more precise, more adaptable, and less expensive than existing bioassays. It has been shown to correlate well at high toxicity levels, and more recently at all levels, with the EPA *Mysid* shrimp protocol and with a method that uses bioluminescent bacteria, in toxicity tests of drilling fluids. It is sensitive to all substances tested that are considered toxic. The novel procedure is insensitive to KCl, to which the *Mysid* shrimp are very sensitive despite its low toxicity; a color-comparator test is being developed for LC50 correlation of muds suspected of containing potassium.

Logistics dictate a day or so to test field samples in the laboratory. The portable tester is ideal for more or less stationary test sites, like industrial plants or drilling rigs, where tests can be completed in a few hours. The portable unit can be used now in the field, and a reagent is being developed to make testing even more flexible and almost instantaneous.

With either its lab equipment or its portable tester, the novel procedure is a quick, inexpensive toxicity rangefinder for drilling fluids.

NOMENCLATURE

Terms not defined in text:

C centigrade
cm centimeter
EC50 toxic concentration that quenches half the light
LC50 toxic concentration that kills half the test organisms in a standard time
LED light emitting diode
ml milliliter
ppm parts per million
pH acidity measurement
ul microliter
vol volume
wt weight

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b. #4,950,594 (1990) *Microbiological Assay Using Bioluminescent Organisms*
c. # 5,130,251 (1992) *Stress-Resistant Bioluminescent Organisms*
d. #5,143,545 (1992) *Antifouling Marine Coatings*
e. #5,192,667 (1993) *Method for Evaluating Anti-fouling Paints*

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Sample Number	Bioluminescent Inhibition, % Dilution 3	Dilution 6	Bioluminescence	Shrimp Assay
OCS6 7750*	ND	ND	-	-
GC2M7**	19 7	20.7	+	+
40¹	ND	ND	-	-
45¹	ND	18.6	+	+
8448701¹	14.0	21.2	+	+
0428702¹	17.3	28.3	+	+
48¹	ND	ND	-	-
41¹	ND	ND	-	-
0118702**	ND	ND	-	-
0258702¹	21.8	26.9	+	+
0228701¹	33 0	26.0	+	+
1360*	ND	ND	-	-
1366*	ND	ND	-	-

Fig. 1: Qualitative correlation of novel method with *Mysid* shrimp test (from Ref. 2)

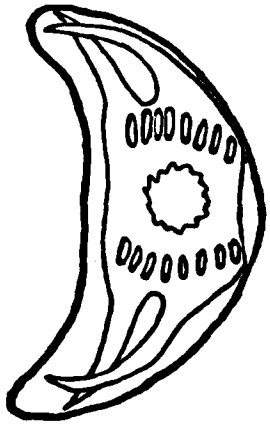

Fig. 2: *Pyrocystis lunula* - long axis is 100 microns (from Ref. 4)

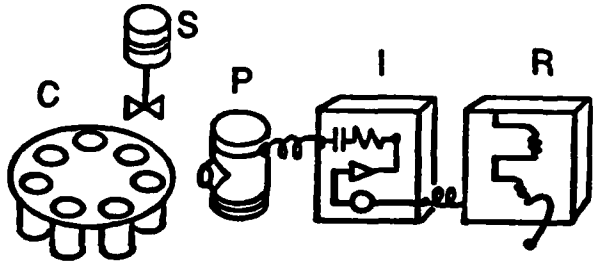

Fig. 3: Lab apparatus for novel method (from Ref. 4)

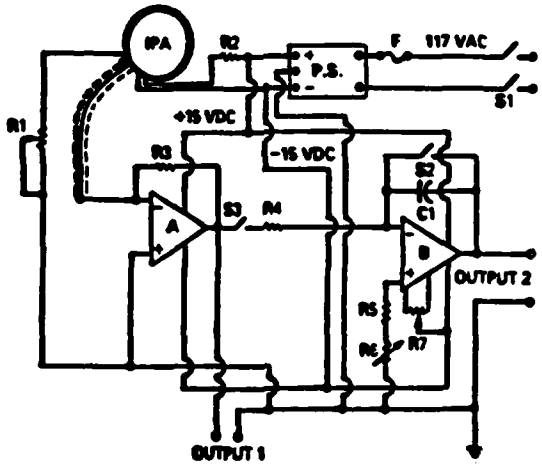

Fig. 4: Patented light-integrating circuit (from Ref. 5. a.)

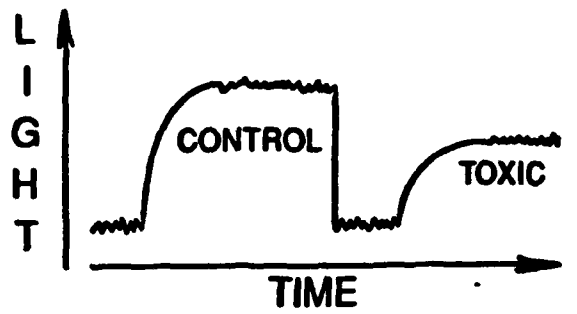

Fig. 5: Cumulative curves, strip-chart recorder (from Ref. 4)

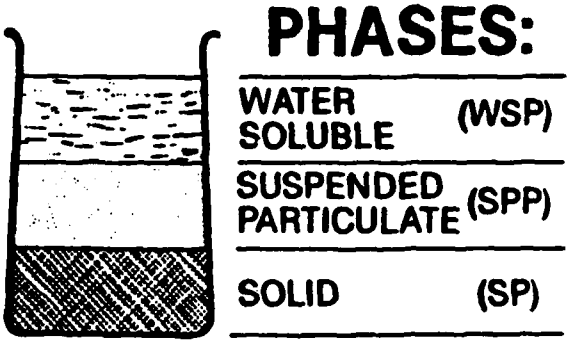

Fig. 6: Drilling mud separation (from Ref. 4)

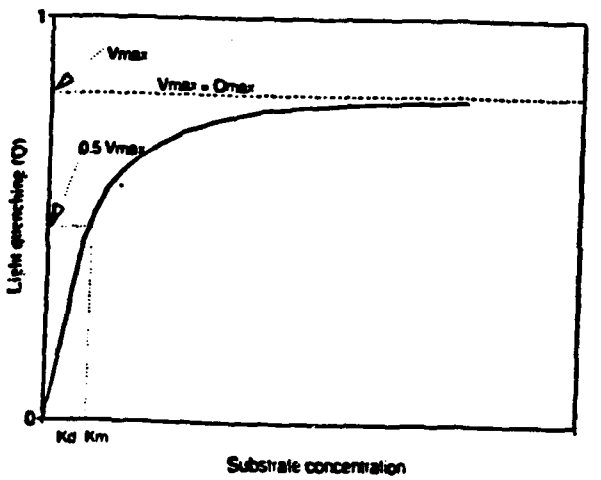

Fig. 7: Enzymatic dose-response curve (from Ref. 7)

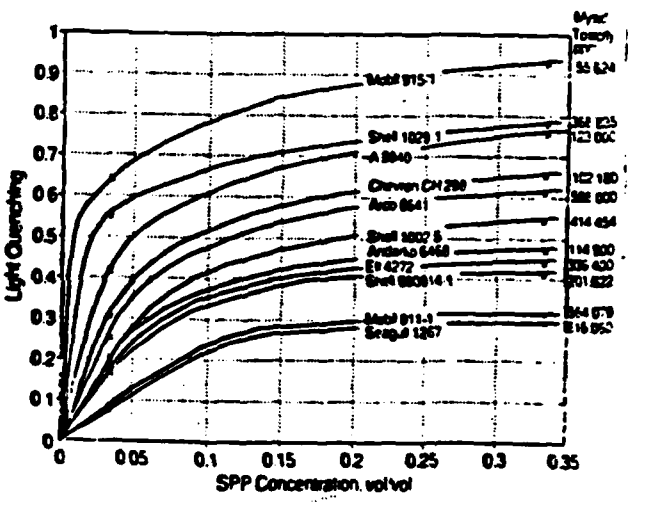

Fig. 8: Mud test results using novel method (from Ref. 7)

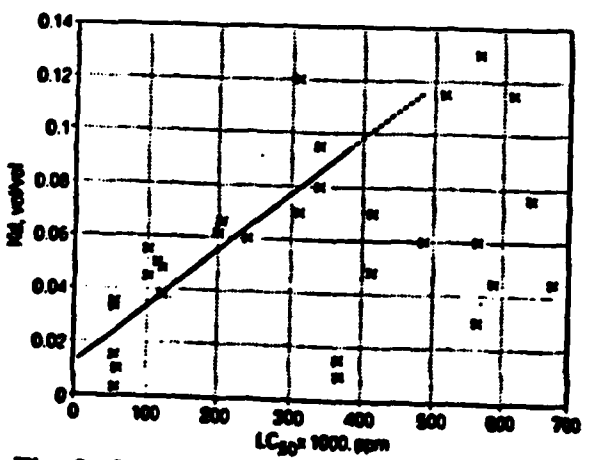

Fig. 9: Correlation of novel method with *Mysid* shrimp LC50 (from Ref. 7)

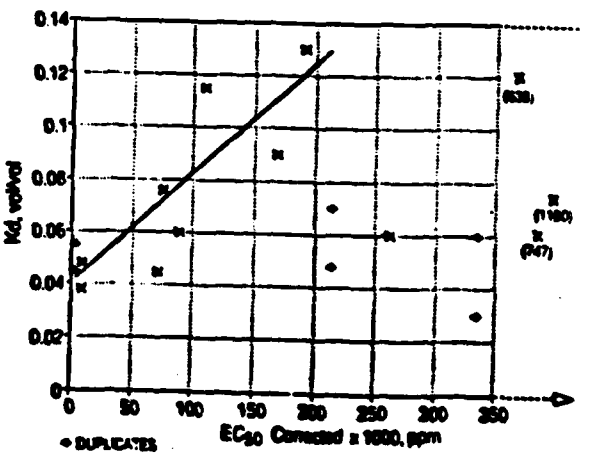

Fig. 10: Correlation of novel method with bioluminescent bacteria test (from Ref. 7)

Mud #	Type	Additive	#/bbl	E50	E25	E10	RSD	LC_{50}
1	Generic	None	None	15.3	3.5	5.2	6.6	>1 MM
2	Field	?	?	44.0	19.5	8.2	12.5	496,000
3	Lubricant	Veg. ester	15	63.4	73.7	75.5	13.1	24,000
4	Sh. Inhib.	KCl	40	36.0	21.3	3.2	7.8	40,400
5	Defoamer	Surfactant	0.125	61.9	62.2	61.9	10.3	198,600
6	Thinner	Polymer	6	60.1	51.2	25.5	11.0	454,600

Fig. 11: Tabulation, results of novel method and cooperative *Mysid* shrimp test on same samples

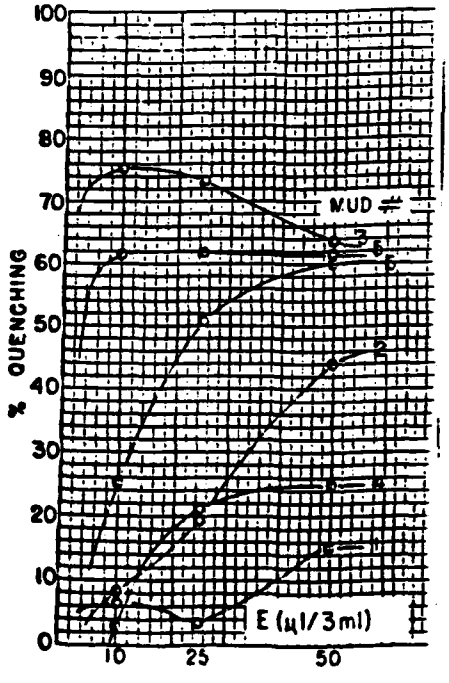

Fig. 12: Graphic results of novel method

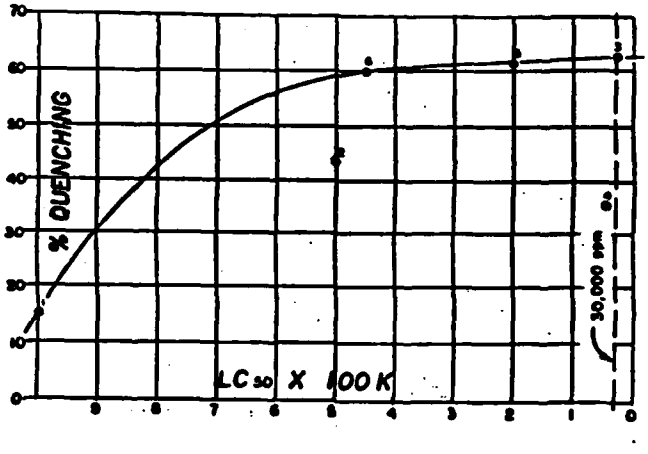

Fig 13: Correlation of novel method with cooperative *Mysid* shrimp test

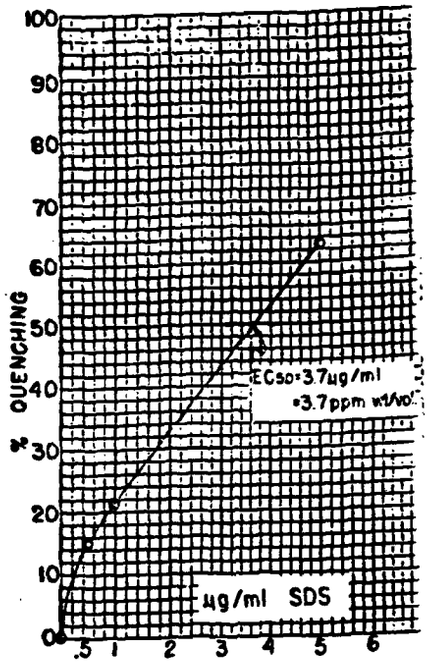

Fig. 14: *Pyrocystis lunula* / SDS dose response curve; EC50 = 3.7 ppm

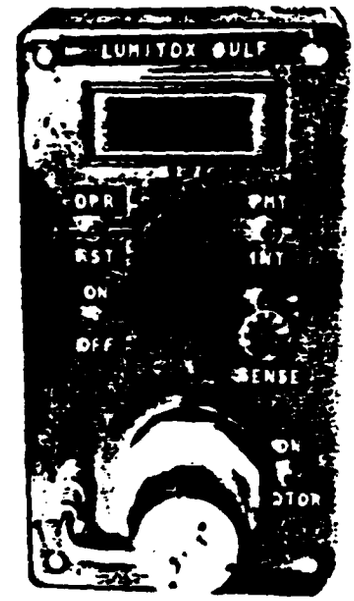

Fig. 15: Hand-held, portable tester

Cells / ml	Cumulative light	Quenching %	RSD	Toxic equivalent
100	199	0 (control)	11	Non-toxic
50	110	45	10	Marginally toxic
25	37	82	21	Extremely toxic

Fig. 16: Tabulation, test results of hand-held, portable toxicity tester

SUPPLEMENTARY

INFORMATION

DEPARTMENT OF THE ARMY
U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
FORT DETRICK, FREDERICK, MD 21702-5012

REPLY TO
ATTENTION OF

ERRATA
AD-B181660

MCMR-RMI-S (70-1y) 3 Apr 96

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limited distribution statement on technical report written for Contract Number DAMD17-93-C-3093. Request the limited distribution statement for Accession Document Number **ADB181660** be changed to "Approved for public release; distribution unlimited." A copy of this report should be released to the National Technical Information Service.

2. Point of contact for this request is Mrs. Judy Pawlus at DSN 343-7322.

for GARY R. GILBERT
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Deputy Chief of Staff
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